comes on in pro-T cells and will enable the deletion of Notch1 target genes in pro-T cells to assess their role in Notch1-induced lineage fate.

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Programming Perpetual T Helper Cell Plasticity

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In this issue of *Immunity*, Lee et al. (2009) and Wei et al. (2009) each investigate the stability of T helper cell lineages and find that commitment to these fates is more plastic than previously appreciated.

Early studies described $CD4^+$ T cell effector lineage commitment as a unidirectional process leading to the stable expression of one of two mutually exclusive cytokine profiles by Th1 and Th2 cells. However, the description of additional helper T cell fates has complicated this model, and in vivo observations of cells with mixed phenotypes have raised questions about the stability of helper lineage fidelity and the relationships among lineages. For example: naive $CD4^+$ T cells differentiate in vitro into Th1 and Th17 effectors that produce IFN-γ and IL-17 in a mutually exclusive manner (at least in the mouse), but these two cytokines are often coexpressed in vivo in the context of infectious or autoimmune diseases (McGeachy and Cua, 2008); Th17 cells can express the regulatory T cell-specifying transcription factor Foxp3, whereas regulatory T cells can be induced to produce IL-17 (Xu et al., 2007; Yang et al., 2008); and regulatory T cells can be diverted to a Th2 cell lineage if Foxp3 expression is suppressed (Wan and Flavell, 2007). Approaching the question of lineage fidelity from two very different angles, the papers of Wei et al. (2009) and Lee et al. (2009) demonstrate that T helper cell lineages retain a surprising degree of plasticity, which may allow them to adopt alternative fates or to acquire functions normally restricted to an opposing $CD4^+$ T cell lineage.

Transcription factors can directly activate or repress gene expression and can induce modifications to chromatin and methylation of DNA at regions where they bind. These epigenetic modifications in turn influence the ease with which transcription factors can bind to their cognate regulatory sequences and (along with differences in transcription factor availability) determine when and to what extent specific genes are expressed in a particular cell. Epigenetic modifications can be inherited through successive cell divisions, but unlike alterations to the underlying DNA sequence, they are also subject to revision in response to changes in environmental cues. There is considerable support for the notion that epigenetic modifications contribute to the heritability of T helper cell lineage choice, while implicitly providing the option for this choice to be subsequently revised.

With this premise in mind, Wei et al. (2009) set out to explore lineage relationships, commitment, and potential for plasticity in T helper cells. To do so, they used ChIP-Seq (a technique they helped to pioneer) to generate complete, comparative genome-wide maps of two informative and complementary histone modifications in naive, Th1, Th2, Th17, and induced (iTreg) and natural regulatory (nTreg) $CD4^+$ T cells. Trimethylation of lysine 4 on histone H3 (H3K4me3) is a permissive mark found at active or poised promoters, whereas trimethylation of lysine 27 on histone H3 (H3K27me3) is a mark of Polycomb-mediated gene silencing. As expected, enrichment for H3K4me3 correlated perfectly with lineage-appropriate expression of the genes encoding the cytokines IFN-γ, IL-4, IL-17A, and IL-17F and the transcription factors ROR(γ)t and Foxp3, which help to instruct Th17 and Treg cell lineage choice, respectively (Figure 1). However, there was considerable heterogeneity as to whether cytokine or transcription factor genes whose expression is specific for one effector lineage were marked by repressive H3K27me3 in opposing lineages. This

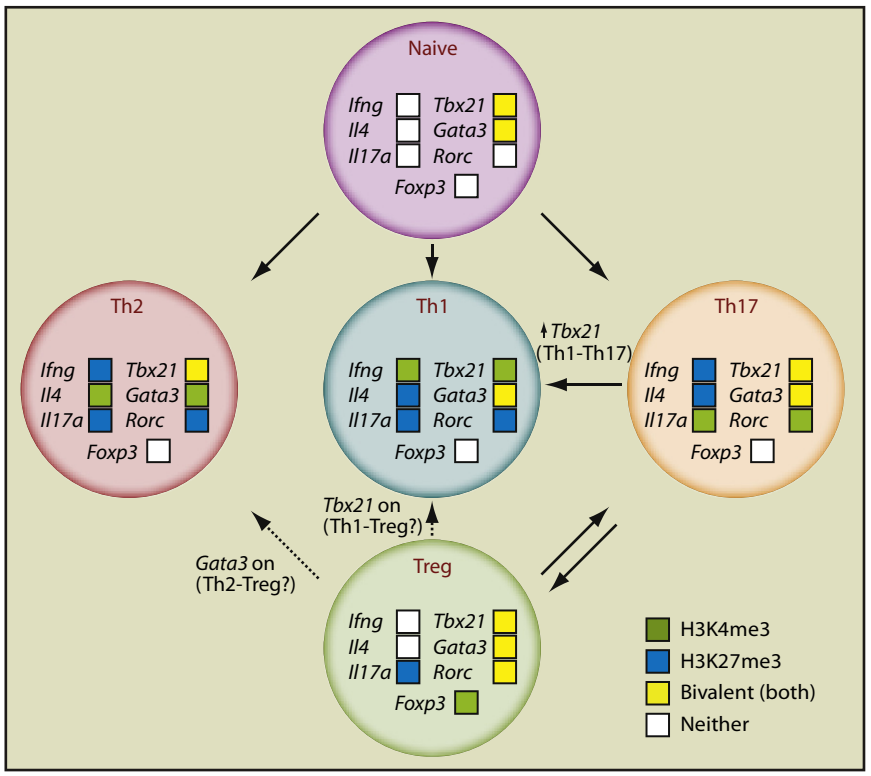

Figure 1. Plasticity and Reciprocity in CD4$^+$ T Cell Lineage Relationships
In additional to the established relationship between naive CD4$^+$ T cell precursors and Th1, Th2, and Th17 T helper cell subsets, recent findings suggest a more plastic set of relationships that allow cells to adopt overlapping functional profiles or potentially to switch from one lineage to another. This plasticity may be facilitated by maintaining transcriptional regulators, such as Tbx21 and Gata3, in a poised, bivalent epigenetic state (i.e., permissive H3K3me3 plus repressive H3K27me3 marks) in opposing lineages, allowing them to be activated in response to future changes in environmental contexts.

finding may suggest the use of alternate mechanisms to silence gene expression, such as DNA methylation or H3K9 methylation, or the intentional absence of silencing in order to retain the potential for future expression.

Intriguingly, *Tbx21* and *Gata3,* which encode the Th1 and Th2 cell lineage-specifying transcription factors T-bet and GATA3, respectively, were marked by H3K4me3 in each of these CD4$^+$ T cell subsets (Figure 1). H3K4me3 was more abundant at *Gata3* in Th2 and at *Tbx21* in Th1 cells, and repressive H3K27me3 was absent, whereas in Th17 cells and in Treg cells, these genes were marked by H3K4me3 plus H3K27me3. Genes whose promoters are marked by these opposing histone modifications are referred to as "bivalent" and are thought to be poised for subsequent activation or silencing. Although it is possible that the retention of *Tbx21* and *Gata3* in a poised bivalent state in Th17 cells and iTreg cells reflects incomplete differentiation of these cells over 10 days in polarizing cultures, this is clearly not the case for nTreg cells. Further, Wei et al. (2009) demonstrate the potential for "lineage reprogramming" in nTreg cells, a fraction of which began to express IFN-γ within 72 hr after stimulation in the presence of IL-12.

But what is the raison d'etre for this plasticity? Are Treg cells programmed this way so that they can serve as surrogate Th1 cells, or might they retain the potential to turn on T-bet, GATA3, or other lineage-restricted transcriptional regulators in order to gain access and thrive at sites where they help to suppress untoward Th1 and Th2 responses? Recent work from the Rudensky laboratory suggests that the latter may be the case—Treg cells must express IRF4, which like GATA3 is essential for Th2 effector differentiation, in order to suppress Th2-mediated immunopathology (A. Rudensky, personal communication).

When first described, the IL-17-producing CD4$^+$ T cell subset was thought to derive from Th1 cells, thus providing evidence for functional plasticity within the Th1 cell lineage. This notion was later abandoned when Th17 cells were shown to develop in the absence of T-bet or STAT4. What remained unexplained was why mice lacking these transcription factors are protected from autoimmune diseases in which Th17 cells are thought to be centrally involved. The work by Lee et al. (2009) addresses this conundrum and the stability of Th17 cell lineage commitment. By using Th17 cells purified based on their expression of an IL-17F-Thy1.1 reporter, they found that Th17 cells can give rise to cells expressing IL-17 plus IFN-γ (Th17-Th1 cells) or IFN-γ alone. Even after 4 weeks in polarizing culture conditions, Th17 cells required the constant presence of TGF-β to sustain expression of IL-17F and IL-17A, to silence IFN-γ, and to dampen yet never silence T-bet and IL-12Rβ2. Moreover, IL-12 could suppress the entire Th17 transcriptional program and induce the Th1 program, over time resulting in a population of cells functionally indistinguishable from Th1 cells derived directly from naive precursors. And in the absence of TGF-β, IL-23 caused a similar Th17-to-Th1 shift, with some notable differences, namely lack of IL-22 and RORα suppression and with slower kinetics.

Lee et al. (2009) did not address underlying epigenetic mechanisms, but as noted above, Wei et al. (2009) found that *Tbx21* is in a bivalent, poised state in Th17 cells, whereas the *Ifng* locus is broadly marked with repressive H3K27me3. Thus, the common feature of Th17 cells and nTreg cells found to correlate with their residual ability to express IFN-γ was the poised status of *Tbx21*, not the state of the *Ifng* locus. Thus, it would be of interest to track changes in epigenetic profiles of Th17 cells as they acquire *Ifng* expression to see whether this and other Th1 loci undergo complete remodeling such that they are indistinguishable from true Th1 cells, or whether such cells retain evidence of having once been Th17 cells. Interestingly, the results of Wei et al. (2009) also provide a suggestion as to why the evolution of Th17 cells into Th17-Th1 or Th1 cells observed by Lee et al. (2009) was not reciprocated—both the *Il17a* and *Rorc* loci were broadly marked with repressive H3K27me3 in Th1 cells.

The Th17 plasticity demonstrated in these two reports comes from cells generated in canonical culture conditions in vitro during which they were constantly exposed to instructive cytokine signals. In vivo, cells likely receive additional signals both favoring and opposing Th17 differentiation, and the nature and relative abundance of these signals, is inconstant. Thus, an important question is whether Th17 cells generated in vivo exhibit the same instability. The evidence at present is both limited and conflicting. By contrast to Lee et al. (2009) who purified Th17 cells based on their expression of an IL-17F-Thy1.1 reporter, Lexberg et al. (2008) purified cells from unmanipulated mice based on their ability to secrete IL-17A directly ex vivo and found that the fraction of cells producing IL-17A alone or along with IFN-γ was relatively stable in the absence of added cytokines for 6 days. Similarly, when human Th17 cells were purified from blood based on their ability to secrete IL-17A directly ex vivo (Streeck et al., 2008), production of IL-17A declined and IFN-γ increased by 6 weeks but were considerably more stable than observed by Lee et al. (2009) whereas Annunziato et al. (2007) found that human Th17 cells cloned directly ex vivo in non-polarizing conditions can be readily induced to produce substantial amounts of IFN-γ when activated in the presence of IL-12. Thus, further study will be required to reconcile these differences, to determine whether cells that can produce IL-17A directly ex vivo represent a relatively more committed subset of Th17 cells, and to explore the underlying mechanisms.

In any case, the two reports in this issue of *Immunity* indicate that the ability of $CD4^+$ T cell subsets to adopt alternate or overlapping fates is determined not simply by the epigenetic states of cytokine loci but by the epigenetic states of the entire set of genes associated with these lineages. Although the data of Wei et al. (2009) characterized only two histone modifications, their multilineage comparison provides hints that global mapping of histone modifications may be predictive of lineages that are most susceptible to deviation and toward which lineages they are most likely to be reprogrammed. In the near future, we can hope to see ever more complete epigenomic and gene expression profiles in T helper cell lineages. These profiles along with data from repolarization studies as carried out by Lee et al. (2009) should help to unravel the many unanswered question regarding T helper cell lineage commitment, plasticity, and overlapping programs of lineage-restricted gene expression.

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Interleukin-17A and Interleukin-17F: A Tale of Two Cytokines

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In this issue of *Immunity*, Ishigame et al. (2009) show that interleukin-17A (IL-17A) mediates autoimmunity whereas both IL-17A and IL-17F are required for mucosal immunity. IL-17A may be more pathologic by inducing proinflammatory cytokines.

Charles Dickens's epic tale of Paris and London begins with "It was the best of times, it was the worst of times," emphasizing the recent advances in wisdom that coincided with a time of great ignorance. In the past 10 years, our understanding of interleukin-17 (IL-17) in mucosal immunity and autoimmunity has greatly expanded. IL-17A, the founding member of the IL-17 family of cytokines, is produced by a subset of $CD4^+$ T cells termed Th17 cells. IL-17A is potent inducer of antimicrobial peptides as well as neutrophil growth factors such as granulocyte colony-stimulating factor (G-CSF) and it